UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/804,969	03/19/2004	Alan L. Greener	25436/2372	7873

27495 7590 11/22/2006

PALMER & DODGE, LLP
KATHLEEN M. WILLIAMS / STR
111 HUNTINGTON AVENUE
BOSTON, MA 02199

EXAMINER	
PETERSEN, CLARK D	
ART UNIT	**PAPER NUMBER**
1657	

DATE MAILED: 11/22/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/804,969	GREENER, ALAN L.	
	Examiner	Art Unit	
	Clark D. Petersen	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-29 are pending in the instant application.

Claims 1-29 were examined on their merits.

Biological Deposit Requirement

Claims 26-29 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The application discloses E. coli strain 209K15 that is encompassed by the definitions for **biological material** set forth in 37 C.F.R. § 1.801. Because it is apparent that this biological material is essential for practicing the claimed invention, it must be obtainable by a reproducible method set forth in the specification or otherwise be known and readily available to the public as detailed in 37 C.F.R. §§ 1.801 through 1.809.

It is unclear whether this biological material is known and readily available to the public or that the written instructions are sufficient to reproducibly construct this biological material from starting materials known and readily available to the public. Accordingly, availability of such biological material is deemed necessary to satisfy the enablement provisions of 35 U.S.C. § 112. If this biological material is not obtainable or available, the requirements of 35 U.S.C. § 112 may be satisfied by a deposit of the biological material. In order for a deposit to meet all criteria set forth in 37 C.F.R. §§

1.801-1.809, applicants or assignee must provide assurance of compliance with provisions of 37 C.F.R. §§ 1.801-1.809, in the form of a declaration or applicant's representative must provide a statement. The content of such a declaration or statement is suggested by the enclosed attachment. Because such deposit will not have been made prior to the effective filing date of the instant application, applicant is required to submit a verified statement from a person in a position to corroborate the fact, which states that the biological material which has been deposited is the biological material specifically identified in the application as filed (37 C.F.R. § 1.804). Such a statement need not be verified if the person is an agent or attorney registered to practice before the Office. Applicant is also reminded that the specification must contain reference to the deposit, including deposit (accession) number, date of deposit, name and address of the depository, and the complete taxonomic description.

In the instant case, applicant includes a reference in the specification including deposit number, date of deposite, and the name of the depository (see, for example, p. 14 of the instant specification, lines 15-23). However not all of the requirements listed in the attachment below have been met. In particular, it is not apparent in the instant application that applicant has made filed a form such as described in the attachment below (see following page), meeting the listed requirements.

SUGGESTION FOR DEPOSIT OF BIOLOGICAL MATERIAL ATTACHMENT

A declaration by applicant or assignee, or a statement by applicant's agent identifying a deposit of biological material and averring the following may be sufficient to overcome an objection or rejection based on a lack of availability of biological material. Such a declaration:

1. Identifies declarant.

2. States that a deposit of the material has been made in a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. The depository is to be identified by name and address. (See 37 C.F.R. § 1.803).

3. States that the deposited material has been accorded a specific (recited) accession number.

4. States that all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of the patent. (See 37 C.F.R. § 1.808(a)(2)).

5. States that the material has been deposited under conditions that assure that access to the material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. § 1.14 and 35 U.S.C. § 122. (See 37 C.F.R. § 1.808(a)(1)).

6. States that the deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. See 37 C.F.R. § 1.806).

7. That he/she declares further that all statements made therein of his/her own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant patent application or any patent issuing thereon.

Alternatively, it may be averred that deposited material has been accepted for deposit under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (e.g., see 961 OG 21, 1977) and that all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent.

Additionally, the deposit must be referred to in the body of the specification and be identified by deposit (accession) number, date of deposit, name and address of the depository, and the complete taxonomic description.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 11, 12, 17, 22, 23, and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Vehmaanpera (FEMS Microbiol Lett, 1989). Vehmaanpera teaches a method of preparing electroporation competent cells by growing them in a hyperosmotic medium. Specifically, the growth medium is LB containing 250 mM sucrose and 50 mM potassium phosphate; the addition of potassium phosphate in particular makes the growth medium hyperosmotic in terms of total salt concentration (see Buffers and Media, p. 166, col. 1, for example). Addition of either glycine or 50 mM potassium phosphate resulted in a 10 fold increase in transformants (see p. 166, col. 2, for example). Part of the process of making the cells electrocompetent involves washing them with an aqueous solution containing 10% glycerol (see Buffers and Media, p. 166, col. 1, for example). The cells are grown in LB broth, which contains tryptone, which is another term for casein hydrolysate (see Materials and Methods, p. 166, col. 1, for example). The cells are induced to take up by electroporation a plasmid that contains an exogenous antibiotic resistance gene and to express that gene, reading on claims 22 and 23. Additionally the cells are separated by virtue of their expression of the antibiotic resistance gene, by growing them on media containing an appropriate antibiotic. Those cells expressing the gene persist, while those that were not

transformed die, reading on claim 24. Therefore the teachings of Vehmaanpera are deemed to anticipate the instant claims 1, 2, 11, 12, 17, 22, 23, and 24.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, and 4-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jessee et al (US 6,960,464 B2) in view of Xue et al (J Microbiol Meth, 1999) and Tunnacliffe et al (WO 98/24882, published 11 June 1998).

Jessee et al teach a method for generating storage-stable competent cells that comprises freeze drying bacteria. Specifically these cells are are lyophilized allowing them to be stored up to room temperature without appreciably losing transformation efficiency (see Summary of the Invention, cols. 1 and 2). The cells can be *E. coli* cells, which are gram negative (see col. 4, lines 19-30, for example). Cells are grown and then collected and resuspended in "a competence buffer. A competence buffer is any solution that enables cells to take up and establish exogenous DNA" (see col. 5, lines 24-26, for example). Additionally the cells are contacted in the course of preparation with a solution comprising 10% glycerol (see Example 1, col 8 line 66 to col 9 line 3, for example). Additionally the process can comprise adding sorbitol as a cryoprotectant (see col. 6, lines 1-13, for example). They then lyophilize the cells, and demonstrate

that upon rehydration the cells retain transformation competence (see col. 9, lines 15 – 63, for example). The growth protocol comprises growing the bacterial cells to an OD_{550} of 0.5 before harvesting (see col. 8, lines 46-50, for example). As stated above, Jessee et al teach that their method of lyophilizing competent cells results in cells that can be transformed by adding a nucleic acid and selecting for transformants. It is well known in the art that *E. coli* can be transformed with a plasmid that confers upon the *E. coli* the ability to produce a polypeptide that the cells would not naturally produce. In the process of culturing transformants, cells that have taken up the pUC19 plasmid are separated from those that have not by growing on medium containing ampicillin, a standard method for evaluating transformation efficiency in the art (see col. 9, lines 35-52, for example), reading on claims 22, 23, and 24.

Jessee et al do not *expressly* teach that the cells prepared by their method are electrocompetent, only that their method is compatible with any solution that can be used in a method of inducing cells to take up exogenous DNA. Jessee et al do not expressly teach growth of competent cells in hyperosmotic media, in which specifically salt concentrations are elevated. Jessee et al do not expressly teach that their method increases the electrotransformation efficiency of cells. Jessee et al do not expressly teach the exact growth conditions recited in the instant claims.

Xue et al teach a method of improving the efficiency of electro-transformation of bacterial cells. They teach that increasing the osmolarity of the growth medium increases the survival of bacterial cells during the process of electroporation. They note that inclusion of 0.5M sorbitol in the LB growth medium led to a 14-fold increase in the

transformation efficiency of the bacteria (see p. 187, col. 2, for example). Xue et al teach that higher electro-transformation efficiencies can be achieved when cells are prepared for electroporation by soaking in electroporation medium containing 10% glycerol, as well as 0.5M sorbitol; this solution is aqueous. The bacterial cells are transformed by electroporation after growth in the hyperosmotic medium with a plasmid containing a kanamycin cassette. After transformation cells are plated on a selective media containing kanamycin; cells that have not been transformed cannot grow, and thus transformed bacteria are separated from untransformed bacteria. Xue et al teach a hyperosmotic growth media containing sorbitol as the "osmoticum"; however, they note that many different solutes can be used as the osmoticum to induce hyperosmolarity (see p. 187, for example) and although it is not clear if the effect in their experiments is due to sorbitol, they note that the increased electroporation efficiency may be a general effect of hyperosmotic growth media (see p. 187 for example).

Tunacliffe et al teach a method of lyophilizing prokaryotic cells, specifically *E. coli*. Tunacliffe et al teach that one can grow *E. coli* in such a manner that the growth conditions induce the cells to produce trehalose, a sugar that protects the cells against adverse conditions such as insufficient hydration or extreme cold. To induce the cells to produce trehalose, the cells are grown in a hyperosmotic medium, and an ideal solute is NaCl, at a concentration of 200 mM above isoosmotic (see p. 10, lines 16-27, for example). Additionally Tunacliffe et al teach that it is well known in the art to reduce oxygen levels of bacterial cultures to induce them to produce trehalose as a protectant against dessication and cold (see p. 10, lines 6-8, for example). Instant claim 10 is also

rejected because it is common practice to grow bacteria at a 1-fold reduction in oxygen, i.e., normal ambient oxygen.

Regarding the addition of casein hydrolysate and maltose, NZY-maltose broth is well known in the art as a rich medium for *E. coli* growth, and typically contains approximately 1% casein hydrolysate and 0.2% maltose. Adjusting the casein hydrolysate to 1.1 or 1.2% would be yielded by result effective adjustment.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to make electrocompetent, lyophilized cells according to the method of Jessee et al using a hyperosmotic medium as taught by Xue et al, because Jessee et al teach that it is possible to lyophilize *E. coli* without compromising their transformation competency, after which they can remain competent, and Xue et al teach that in fact, high osmolarity media are ideal for preparing electrocompetent cells that are actually more competent than if they were grown in isoosmotic medium.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ the methods of Tunacliffe et al for lyophilizing bacteria in a method of making storage-stable competent bacteria taught by Jessee et al, because Jessee et al teach that it is desirable to lyophilize competent bacteria to make them stable for long periods of time at higher temperatures, and Tunacliffe et al teach that is possible to effectively lyophilize bacteria by growing them in high salt/low oxygen concentrations to induce them to produce trehalose which acts as a cell stabilizer during the lyophilization process.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use NZY-maltose broth as a growth medium, because it is well known in the art to provide a rich broth for certain catabolic applications, such as inducing bacteria to produce polypeptides or sugars such as trehalose.

One would have been motivated make electrocompetent, lyophilized cells according to the method of Jessee et al using a hyperosmotic medium as taught by Xue et al, because so for the expected benefit that, not only would the method allow one to prepare cells that are stable for longer periods at higher temperatures, but that in fact the cells would be more efficient at taking up DNA than if they were grown in isoosmotic medium.

One would have been motivated employ the methods of Tunacliffe et al for lyophilizing bacteria in a method of making storage-stable competent bacteria taught by Jessee et al, because so for the expected benefit of creating competent bacteria that are more resistant to higher storage temperatures.

One would have been motivated to use NZY-maltose broth as a growth medium for the expected benefit of giving the bacteria more nutrients to produce more of the desired biological molecule.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jessee et al (US 6,960,464 B2) in view of Tunnacliffe et al (WO 98/24882, published 11

June 1998) and Greener et al (US 6,040,184, issued 21 Mar, 2000). The teachings of Jessee et al and Tunnacliffe et al are discussed above and applied as before.

Neither Jessee et al nor Tunnacliffe et al expressly teach a cell that has an electrotransformation efficiency at least 30% greater than that for cells of the same bacterial strain grown under conditions of isoosmotic salt, and neither reference expressly teaches cells with an electrotransformation efficiency of at least 2 X 10^{10} cfu/μg DNA.

The instant specification includes Example 1 (p.18). In this example, the electrotransformation efficiency of Electroten Blue cells is compared when the cells are grown under standard conditions versus conditions of high salt and limited oxygen. The table in instant Example 1 teaches that Electroten Blue cells are transformed approximately 33% more efficiently under the high salt/low oxygen conditions, and the absolute transformation efficiency is 2.0 X10^{10} cfu/μg. Stratagene product literature states that Electroten cells are covered by US Patents #6,635,457, #6,586,249, and #6,040,184 (see Stratagene product literature entitled Strategies, vol. 18, no. 4, for example). US Patent 6,040,184 discloses an *E. coli* strain with an electrotransformation efficiency of 1.6 X 10^{10} cfu/μg DNA when exposed to 2.5% sorbitol (see Table 2, col. 7, for example). Example 1 of the instant specification, which reads on instant claims 2 and 3, relies for enablement on a strain of bacteria already covered by US Patent 6,040,184. Although US Patent 6,040,184 does not disclose a method of growing the particular strain in high salt/low oxygen, it is an inherent property of the bacteria that when grown in such conditions it has a electrotransformation efficiency of 2.0 X 10^{10}

cfu/μg DNA and that its electrotransformation efficiency is at least 30% greater than when grown under standard conditions, and these properties are exemplified in the instant specification.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ Stratagene Electroten-Blue electrocompetent cells in a method of preparing lyophilized electrocompetent cells taught by Jessee et al and Tunnacliffe et al, because Jessee et al teach that competent cells can be lyophilized, Tunnacliffe et al teach a reliable method of lyophilizing bacteria, and Greener et al teach a highly transformable electrocompetent cell line. One would have been motivated to do so for the expected benefit that lyophilized cells are much easier to store – i.e. they require no special cooling – and that the cells taught by Greener et al would be extremely sensitive in a transformation protocol, allowing successful transformation when available DNA is limited.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have had a reasonable expectation of success in practicing the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Clark D. Petersen whose telephone number is (571)272-5358. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on (571)272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

CDP
9/30/2006

JON WEBER
SUPERVISORY PATENT EXAMINER